Chapter 7
The Behavior of Proteins: Enzymes, Mechanisms, and Control

SUMMARY

Section 7.1

- Allosteric enzymes exhibit different behaviors compared to non-allosteric enzymes, and the Michaelis-Menten equations are not applicable.
- A plot of velocity vs. [S] for an allosteric enzyme has a sigmoidal shape.
- One type of control often seen with allosteric enzymes is called feedback inhibition.
- Inhibitors and activators can control the activity of an allosteric enzyme.

Section 7.2

- The two principal models for allosteric enzyme behavior are called the concerted model and the sequential model.
- In the concerted model, the enzyme is thought of as being in a taut form, T, or a relaxed form, R. All subunits are found in one or the other, and there is an equilibrium between the T and R forms.
- Substrate binds more easily to the R form than to the T form, inhibitors stabilize the T form, and activators stabilize the R form
- In the sequential model, subunits of the enzyme can change sequentially from the T form to the R form and back again.
- Binding of one molecule of substrate to one subunit stimulates the transition of the subunit to the R form, which then stimulates another subunit to change to the R form.
- Binding of inhibitor to one subunit induces a change in the other subunits to a form with lower affinity for the substrate. Binding of an activator to one subunit induces a shift in the other subunits to a form that has a high affinity for substrate.

Section 7.3

- Many enzymes are controlled by phosphorylation.
- Enzymes called kinases use high-energy molecules, such as ATP, to transfer a phosphate to a specific residue in an enzyme.
- These amino acid residues are usually serine, threonine, or tyrosine residues.
- In some cases, phosphorylation increases the activity of an enzyme, while in other cases it decreases it.

Section 7.4

- Zymogens are inactive precursors of an enzyme
- A zymogen is converted to the active form by the irreversible cleavage of specific peptide bonds in the protein.
- Many digestive enzymes, such as trypsin and chymotrypsin, are initially produced as zymogens. They become active only after arriving at their final destination.
- Caspases are an important class of proteases that are involved in cellular processes such as programmed cell death, or apoptosis. They are first produced in an inactive form called procaspases, which are later activated by proteolysis of their immature forms.

Section 7.5

- The unique orientation of the amino acids in the active site promotes the catalysis of a chemical reaction.
- To understand the catalytic mechanism, the critical amino acids in the active site must be determined. Labeling reagents are often used for this purpose.
- Histidine 57 and Serine 195 play the most important roles in the mechanism of chymotrypsin action.

Section 7.6

- Enzymes are known to catalyze familiar organic chemical reactions.
- One of the most common is a nucleophilic substitution reaction, of which there are two principal types – S_N1 and S_N2.
- Other common reactions are general acid-base catalysis and metal-ion catalysis.

Section 7.7

- The transition state of an enzyme-catalyzed reaction represents a structure between the substrates and the products
- Transition state analogs are molecules that are shaped to mimic the transition state. They make potent enzyme inhibitors due to their tight binding to the active site.
- By using a transition-state analog to illicit antibodies, catalytic antibodies can be made, called abzymes. Some abzymes have been made for use in medicine.

Section 7.8

- Coenzymes are nonprotein substances that take part in enzymatic reactions and are regenerated for further reaction.
- Metal ions can serve as coenzymes, frequently by acting as Lewis acids.
- There are also many organic coenzymes, such as NAD^+ and FAD, most of which are vitamins or are structurally related to vitamins.

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LECTURE NOTES

There are two major topics covered in this chapter, and they each deserve a lecture, at least. The first lecture should be devoted to enzyme control mechanisms, especially allosteric enzymes. Covalent control of enzymes fits well here, as many enzymes utilize both control systems. Other mechanistic concepts, such as zymogens are also covered. A second lecture should introduce enzyme mechanisms. Chymotrypsin is a useful model for this. General classes of enzyme action are described, along with an introduction to enzyme cofactors. A good topic that links several concepts is the creation and use of catalytic antibodies, such as those used to help treat cocaine addiction.

LECTURE OUTLINE

I. Behavior of allosteric enzymes
    A. Variance from the Michaelis-Menten model
    B. Control mechanisms
        1. Feedback inhibition
        2. Allosteric effectors (K vs. V systems, homo & heterotropic)
II. Behavioral models
    A. Concerted model
    B. Sequential model
III. Control of enzymes by phosphorylation
IV. Zymogens
V. Nature of active sites
    A. Essential residues
    B. Active site architecture
    C. Chymotrypsin
        1. Labeling essential amino acids
        2. Active site architecture
        3. Mechanism of action
VI. Chemical reactions involved in enzyme mechanisms
    A. Nucleophilic substitutions
    B. General acid-base catalysis
    C. Metal-ion catalysis
    D. Importance of stereospecificity
VII. The active site and transition states
VIII. Coenzymes

ANSWERS TO PROBLEMS

7.1 The Behavior of Allosteric Enzymes

1. Allosteric enzymes display sigmoidal kinetics when rates are plotted versus substrate concentration. Michaelis–Menten enzymes exhibit hyperbolic kinetics. Allosteric enzymes usually have multiple subunits, and the binding of substrates or effector molecules to one subunit changes the binding behavior of the other subunits.
2. It is an enzyme used in the early stages of cytidine nucleotide synthesis.

3. ATP acts as a positive effector of ATCase, and CTP acts as an inhibitor.
4. The term K_M should be used for enzymes that display Michaelis–Menten kinetics. Thus, it is not used with allosteric enzymes. Technically, competitive and noncompetitive inhibition are also terms that are restricted to Michaelis–Menten enzymes, although the concepts are applicable to any enzyme. An inhibitor that binds to an allosteric enzyme at the same site as the substrate is similar to a classical competitive inhibitor. One that binds at a different site is similar to a noncompetitive inhibitor, but the equations and the graphs characteristic of competitive and noncompetitive inhibition don't work the same way with an allosteric enzyme.
5. A K system is an allosteric enzyme in which the binding of inhibitor alters the apparent substrate concentration needed to reach one-half V_{max}, $S_{0.5}$.
6. A V system is an allosteric enzyme in which the binding of inhibitor changes the V_{max} of the enzyme but not the $S_{0.5}$.
7. Homotropic effects are allosteric interactions that occur when several identical molecules are bound to a protein. The binding of substrate molecules to different sites on an enzyme, such as the binding of aspartate to ATCase, is an example of a homotropic effect. Heterotropic effects are allosteric interactions that occur when different substances (such as inhibitor and substrate) are bound to the protein. In the ATCase reaction, inhibition by CTP and activation by ATP are both heterotropic effects.
8. ATCase is made up of two different types of subunits. One of them is the catalytic subunit, and there are six of them organized into two trimers. The other is the regulatory subunit, which consists of six protein subunits organized into three dimers.
9. Enzymes that exhibit cooperativity do not show hyperbolic curves of rate versus substrate concentration. Their curves are sigmoidal. The level of cooperativity can be seen by the shape of the sigmoidal curve.
10. Inhibitors make the shape of the curve more sigmoidal.
11. Activators make the shape of the curve less sigmoidal.
12. $K_{0.5}$ is the substrate concentration that leads to half of the maximal velocity. This term is used with allosteric enzymes, where the term K_M is not appropriate.
13. A mercury compound was used to separate the subunits of ATCase. When the subunits were separated, one type of subunit retained catalytic activity but was no longer allosteric and was not inhibited by CTP. The other subunit type had no ATCase activity, but it did bind to CTP and ATP.

7.2 The Concerted and Sequential Models for Allosteric Enzymes

14. In the concerted model, all the subunits in an allosteric enzyme are found in the same form, either the T form or the R form. They are in equilibrium, with each enzyme having a characteristic ratio of the T/R. In the sequential model, the subunits change individually from T to R.
15. The sequential model can explain negative cooperativity, because a substrate binding to the T form could induce other subunits to switch to the T form, thereby reducing binding affinity.

16. Greater cooperativity is favored by having a higher ratio of the T/R form. It is also favored by having a higher dissociation constant for the substrate binding to the T form.
17. The L value is the equilibrium ratio of the T/R form. The *c* value is the ratio of the dissociation constants for substrate and the two forms of enzyme, such that $c = K_R/K_T$.
18. Many models are possible. We never really know for sure how the enzyme works, rather, we create a model that explains the observed behavior. It is very possible that another model would do so as well.
19. Scientists looked for drugs that would mimic the behavior of signaling molecules, such as hormones and neurotransmitters.
20. Side effects occur because the drug that is meant to effect one type of receptor will likely affect several others unintentionally.
21. First, allosteric effectors modulate the response in a more subtle way than orthosteric ones. Second, using allosteric drugs allows the drug to be more specific for one or a few receptor types. Third, allosteric drugs can be safer because they have no effect unless the natural ligand is present.
22. Valium is an allosteric drug that binds to a different site on the receptors for γ-aminobutyric acid (GABA). Valium turns up the response of the receptor for the GABA. When Valium is bound, the response to GABA goes up many fold.
23. Taking too much Valium is not as deadly as taking too much Phenobarbital as it is not Valium that causes a direct effect, rather it modulates the effect of the bound, natural ligand.
24. One is Amgen's Cincalcet, a drug designed to fight chronic kidney failure by improving the action of calcium receptors. The other is an HIV medication by Pfizer called Maraviroc. It interferes with HIV entry into the cells.

7.3 Control of Enzyme Activity by Phosphorylation

25. A kinase is an enzyme that phosphorylates a protein using a high-energy phosphate, such as ATP, as the phosphate donor.
26. Serine, threonine, and tyrosine are the three most often phosphorylated amino acids in proteins that are acted upon by kinases. Aspartate is another one often phosphorylated.
27. The allosteric effect can be faster because it is based on simple binding equilibrium. For example, if AMP is an allosteric activator of glycogen phosphorylase, the immediate increase in AMP when muscles contract can cause muscle phosphorylase to become more active and to provide energy for the contracting muscles. The phosphorylation effect requires the hormone cascade beginning with glucagon or epinephrine. There are many steps before the glycogen phosphorylase is phosphorylated, so the response time is slower. However, the cascade effect produces many more activated phosphorylase molecules, so the effects are longer and stronger.

28. As part of the mechanism, the sodium–potassium ATPase has an aspartate residue that becomes phosphorylated. This phosphorylation alters the conformation of the enzyme and causes it to close on one side of the membrane and open on the other, moving ions in the process.
29. Glycogen phosphorylase is controlled allosterically by several molecules. In the muscle, AMP is an allosteric activator. In the liver, glucose is an allosteric inhibitor. Glycogen phosphorylase also exists in a phosphorylated form and an unphosphorylated form, with the phosphorylated form being more active.
30. Salicylic acid, which comes from the bark of the willow tree.
31. Salicylate stimulates AMPK, which stimulates fat burning. Researchers believe this effect lowers plasma fatty acids and reduces the risk of heart attacks and type 2 diabetes.

7.4 Zymogens

32. The digestive enzymes trypsin and chymotrypsin are classic examples of regulation by zymogens. The blood-clotting protein thrombin is another.
33. Trypsin, chymotrypsin, and thrombin are all proteases. Trypsin cleaves peptide bonds where there are amino acids with positively charged side chains (Lys and Arg). Chymotrypsin cleaves peptides at amino acids with aromatic side chains. Thrombin cleaves the protein fibrinogen into fibrin.
34. Caspases are a family of homodimer cysteine proteases responsible for many processes in cell biology, including apoptosis, signaling within the immune system and stem cell differentiation.
35. Chymotrypsinogen is an inactive zymogen. It is acted upon by trypsin, which cleaves peptides at basic residues, like arginine. When trypsin cleaves between the arginine and the isoleucine, chymotrypsinogen becomes semiactive, forming π-chymotrypsin. This molecule digests itself further, forming the active α-chymotrypsin. As it turns out, the α-amino group of the isoleucine produced by the first cleavage is near the active site of α-chymotrypsin and necessary for its activity.
36. Zymogens are often seen with digestive enzymes that are produced in one tissue and used in another. If the enzyme were active immediately upon production, it would digest other cell proteins, where it would cause great damage. By having it produced as a zymogen, it can be safely made and then transported to the digestive tissue, such as the stomach or small intestine, where it can then be activated.
37. This allows for a more rapid response when the hormone is needed. The hormone is already synthesized and usually just requires breaking one or two bonds to make it active. The hormone can be poised and ready to go on demand.
38. Apoptosis is a natural phenomenon of programmed cell death.
39. Disruption of apoptosis can lead to forms of cancer and unwanted cell death, such as cells surrounding neurons that have died from a stroke.

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7.5 The Nature of the Active Site

40. Serine and histidine are the two most critical amino acids in the active site of chymotrypsin.
41. The initial phase releases the first product and involves an acylenzyme intermediate. This step is faster than the second part, in which water comes into the active site and breaks the acyl-enzyme bond.
42. In the first step of the reaction, the serine hydroxyl is the nucleophile that attacks the substrate peptide bond. In the second step, water is the nucleophile that attacks the acyl-enzyme intermediate.
43. Histidine 57 performs a series of steps involving general base catalysis followed by general acid catalysis. In the first phase, it takes a hydrogen from serine 195, acting as a general base. This is followed immediately by an acid catalysis step, in which it gives the hydrogen to the amide group of the peptide bond that is breaking. A similar scheme takes place in the second phase of the reaction.
44. The first phase is faster for several reasons. The serine at position 195 is a strong nucleophile for the initial nucleophilic attack. It then forms an acyl-enzyme intermediate. In the second phase, water is the nucleophile, and it takes time for water to diffuse to the right spot to perform its nucleophilic attack. It is also not as strong a nucleophile as the serine. Therefore, it takes longer for water to perform its nucleophilic attack and break the acyl-enzyme intermediate than it takes for serine to create it.
45. Histidine 57 exists in both the protonated and unprotonated form during the chymotrypsin reaction. Its pK_a of 6.0 makes this possible in the physiological pH range.
46. Instead of a phenylalanine moiety (similar to the usual substrates of chymotrypsin), use a nitrogen-containing basic group similar to the usual substrates of trypsin.

7.6 Chemical Reactions Involved in Enzyme Mechanisms

47. They act as Lewis acids (electron-pair acceptors) and can take part in enzyme catalysis mechanisms of enzymes.
48. The carbon of a carbonyl group is often attacked by a nucleophile.
49. General acid catalysis is the part of an enzyme mechanism in which an amino acid or other molecule donates a hydrogen ion to another molecule.
50. S_N1 stands for unimolecular nucleophilic substitution. The unimolecular part means that it obeys first-order kinetics. If the reaction is R:X + Z: → R:Z + X:, with an S_N1 reaction, the rate depends on the speed with which the X breaks away from the R. The Z group comes in later and quickly, compared with the breakdown of R:X. S_N2 stands for bimolecular nucleophilic substitution. This happens with the same reaction scheme if the Z attacks the R:X molecule before it breaks down. Thus, the concentration of both R:X and Z: are important, and the rate displays second-order kinetics.
51. The S_N1 reaction leads to loss of stereospecificity as the X group leaves before the entering nucleophile. This means that the nucleophile can enter from different angles, leading to different isomers.

52. The results do not prove that the mechanism is correct, because results from different experiments could contradict the proposed mechanism. In that case, the mechanism would have to be modified to accommodate the new experimental results.

7.7 The Active Site and Transition States

53. A good transition-state analogue would have to have a tetrahedral carbon atom where the amide carbonyl group was originally found, since the transition state involves a momentary tetrahedral form. It would also have to have oxygens on the same carbon, so that there would be sufficient specificity for the active site.
54. The induced-fit model assumes that the enzyme and substrate must both move and change to conform to each other perfectly. Thus, the true fit is not between the enzyme and substrate but between the enzyme and the transition state of the substrate on its way to product. A transition-state analogue fits the enzyme nicely in this model.
55. An abzyme is created by injecting a host animal with a transition-state analogue of a reaction of interest. The host animal makes antibodies to the foreign molecule, and these antibodies have specific binding points that mimic an enzyme surrounding a transition state. The purpose is to create an antibody with catalytic activity.
56. Cocaine blocks the reuptake of the neurotransmitter dopamine at synapses. Thus, dopamine stays in the system longer, overstimulating the neuron and leading to the reward signals in the brain that lead to addiction. Using a drug to block a receptor would be of no use with cocaine addiction and would probably just make removal of dopamine even more unlikely.
57. Cocaine can be degraded by a specific enzyme that hydrolyzes an ester bond that is part of cocaine's structure. In the process of this hydrolysis, the cocaine must pass through a transition state that changes its shape. Catalytic antibodies to the transition state of the hydrolysis of cocaine hydrolyze cocaine to two harmless degradation products—benzoic acid and ecgonine methyl ester. When degraded, the cocaine cannot block dopamine reuptake. No prolongation of the neuronal stimulus occurs, and the addictive effects of the drug vanish over time.

7.8 Coenzymes

58. Nicotinamide adenine dinucleotide, oxidation–reduction; flavin adenine dinucleotide, oxidation–reduction; coenzyme A, acyl transfer; pyridoxal phosphate, transamination; biotin, carboxylation; lipoic acid, acyl transfer.
59. Most coenzymes are derivatives of compounds we call vitamins. For example, nicotinamide adenine dinucleotide is produced from the B vitamin niacin. Flavin adenine dinucleotide comes from riboflavin.
60. Vitamin B_6 is the source of pyridoxal phosphate, which is used in transamination reactions.
61. Coenzymes can accomplish the same mechanisms that the amino acids do in a reaction. For example, a metal ion may act as a general acid or base. Parts of a coenzyme, such as the reactive carbanion of thiamine pyrophosphate, may act as a nucleophile to catalyze the reaction.

62. Yes, there would be a preference. Because the coenzyme and the other substrate will be locked into the enzyme, the hydride ion would come from some functional group that had a fixed position. Therefore, the hydride would come from one side.
63. Green chemistry refers to the modern techniques that replace large quantities of toxic chemicals previously used with smaller quantities of less toxic chemicals
64. TAML's are used to detoxify natural and synthetic pollutants.